

ABSTRACT

A method and assay system is provided for screening for antimicrobial agents that inhibit or otherwise disrupt the transcription of bacterial aminoacyl-tRNA synthetases, particularly those in Gram-positive bacteria. The method and assay utilizes purified RNA polymerase, isolated, for example, from either *Bacillus subtilis* or *Escherichia coli*, and a template DNA fragment which encodes glycyl-tRNA synthetase, containing the promoter and leader region of the *B. subtilis* *glyQS* gene, including the T-Box terminator/antiterminator. Incubation in the presence of nucleotide triphosphates results in synthesis of an RNA transcript initiating at the *glyQS* promoter and terminating at the leader region terminator. Read-through of the terminator, and synthesis of an extended transcript, is dependent on addition of purified glycyl-tRNA. The method and assay is dependent on the use of the specified tRNA species; non-specific tRNA fails to stimulate antitermination. The assay can be used to screen for inhibitors that specifically inhibit the T-box antitermination mechanism.